

GROWTH RESPONSE OF OKRA (*ABELMOSCHUS ESCULENTUS* (L) MOENCH) TO ARBUSCULAR MYCORRHIZAL FUNGUS INOCULATION IN STERILE AND NON-STERILE SOIL

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ABSTRACT

In a pot experiment, the growth of okra inoculated with *Glomus mosseae* in sterile and non-sterile soil was investigated. Inoculation with *G. mosseae* increased plant growth, fruit yield and nutrient uptake in sterile soil more than in non-sterile soil. Arbuscular mycorrhizal fungi (AMF) colonization was highest in inoculated plants grown in sterile soil and lowest in uninoculated plants grown in non-sterile soil. Foliar nutrient yield was consistently higher in sterile soil inoculated plants than in other treatments. The increased growth in inoculated sterile soil plants is explained in the light of enhanced nutrient uptake by the AMF which could have led to increased chlorophyll synthesis and subsequent increased photosynthesis. On the other hand, the reduced growth of plants in non-sterile soil could have resulted from the negative effect of soil pathogens which either competed with the mycorrhizal fungi for colonization of the okra roots or grazed on the mycorrhizal propagules.

KEYWORDS: Okra, Mycorrhiza, Sterile and Non-Sterile Soil

INTRODUCTION

Okra (*Abelmoschus esculentus*) (L) Moench, is a vegetable crop belonging to the family Malvaceae. It is widely cultivated in the tropics for its young fresh leaves and fruits used in thickening soup as vegetables in many countries of the world (Nwangburuka *et al.*, 2011). In south eastern parts of Nigeria, okra is an important vegetable in their traditional draw soup which is a delicacy among the Efik, Ibibio and Igbo tribes. Okra seeds are good sources of quality edible oil and protein while the whole fruit contains vitamins, minerals such as calcium and potassium, calories and amino acids (Berry *et al.*, 1988). On the whole, okra is regarded as a popular health food as it has high fiber, vitamin C and folate contents (Corleone, 2014). The presence of vitamin C and folate makes it a good source of antioxidants. The stem bark is good for making fibres and ropes. Cultivation of okra in Nigeria and most West African countries is done by small scale farmers with low inputs who often do not get maximum yield possible for this crop in the available marginal soils. Those of them that can afford depend on heavy inorganic fertilizer application to obtain reasonable yield. The use of inorganic fertilizers in most soils of the tropics has been reported to be accompanied by soil acidity problems (Kang *et al.*, 1990).

Arbuscular mycorrhizal fungi (AMF) are known to form symbiotic association with most agricultural crops (Smith and Read, 1997). Their importance in enhancing growth and yield of crops are well documented (Okon, 2004; Yaseen *et al.*, 2011). The plant host supplies the mycorrhizal symbiont with carbon from its photosynthates and benefits from nutrients and water it extracts from the soil in return (Malekzadeh *et al.*, 2007; Thamizhiniyan *et al.*, 2009). Other benefits include alteration of some physiological and biochemical activities which positively affect the

syntheses of growth promoting hormones and chlorophyll (Ayoob *et al.*, 2011), resistance to water stress (Auge *et al.*, 2004) and pathogens (Borowicz, 2001; Muchovej, 2004; Serfoji *et al.*, 2010; Hemavani and Thippeswamy, 2014). One of the well established nutrients uptake associated with AMF colonization in plants is phosphorus (Azcón, 1994; Ebel *et al.*, 1994; Graham 2001). Plants growing in phosphorus deficient soils rely more on AMF (Bationo *et al.*, 2000). Phosphorus is a very important constituent of organic molecules and participates in many metabolic processes in plants. The growth and yield of okra has been demonstrated to be significantly affected by phosphorus levels (Arora *et al.*, 1994; Omotoso and Shittu, 2007). Thus inoculation with appropriate AMF which can extract phosphorus and other nutrients for the crop can be used to increase the production of this crop without much phosphorus fertilizer input thereby protecting the environmental integrity.

Although some studies have been done elsewhere to evaluate the effect of AMF on the growth and yield of okra, no such studies has been documented for the sandy loam soil of Cross River Basin in Nigeria. Thus the purpose of this study was to investigate the effect of *Glomus mosseae* on the growth and yield of okra in sterile and non-sterile soil with a view to using AMF to boost okra production in this part of the tropics.

MATERIALS AND METHODS

Top soil (0-15cm depth) was obtained from the University of Calabar Botanical garden. This was sieved to remove pebbles, root fragments and other non-soil materials. Part of this soil was oven sterilized at 200°C while the remaining was used as non-sterile. A sub-sample of the soil was taken to the laboratory to determine its physico-chemical properties using the method of Juo (1979).

Six ten litres plastic pots were filled with 9 kg of sterile soil each while another batch of six were filled with 9 kg of non-sterile soil. Three pots from each batch were randomly selected and designated arbuscular mycorrhizal fungus inoculated (M^+) while the others were uninoculated (M^-). Ten seeds of short duration variety okra were planted in each pot and thinned out to three seedlings per pot two weeks after germination. The experiment was laid out in a completely randomized design with all treatments replicated three times. Inoculation was done by placing 20g of crude *G. mosseae* inoculum comprising of spores and infected maize root fragments. The non-mycorrhizal treatments were given autoclaved equivalent.

Growth Measurements

Ten weeks after germination, the plants were carefully uprooted after thoroughly watering the soil to loosen it. Each plant was separated into roots, stems, leaves and fruits. These were all placed in separately labeled paper envelopes and oven dried at 70°C to constant dry weight to determine the biomass yield. Subsamples from leaves were taken for nitrogen, phosphorus, potassium and magnesium content analysis using the method of Juo (1979). Foliar nutrient yield was then calculated as the product of the percentage nutrient content and the total biomass yield (Parmar and Sharma, 1996).

Subsamples of fresh feeder roots were taken at the time of harvest for determination of percentage arbuscular mycorrhizal fungi colonization of roots. The feeder roots were thoroughly washed in distilled water and fixed in 50% ethanol. Clearing and staining were carried out following the method of Koske and Gemma (1989). The stained roots were assessed for AMF colonization using the gridline intersect method of Giovannetti and Mosse (1980). Stained roots were spread on a gridline plate and viewed under a dissecting microscope at $\times 45$ magnification.

Statistical Analysis

All data obtained from this investigation were subjected to combined analyses of variance using SAS (SAS, 1996). Duncan's multiple range test was used to separate the means.

RESULTS

The physicochemical analysis of the experimental soil used revealed that physically it was composed of 78.7% sand; 9.0% silt; 12.3% clay; 1.86% organic matter with a sandy loam texture and a pH of 5.69. Chemically, it was made up of 0.08 mg/kg total nitrogen; 85.0mg/kg available P; 1.40cmol kg⁻¹ Ca; 0.8cmol kg⁻¹ Mg and 0.11cmol kg⁻¹ K.

Inoculation with *Glomus mosseae* greatly increased the growth of okra as indicated by biomass and fruit yield (Table 1). Inoculated okra planted in sterile soil gave the highest biomass and fruit yield followed by uninoculated ones planted in sterile soil. Okra planted in non-sterile soil gave a significantly reduced biomass yield irrespective of whether they were inoculated with *G. mosseae* or not. The fruit yield was also reduced here.

The percentage of roots colonized by arbuscular mycorrhizal fungus (AMF) was highest in inoculated okra planted in sterile soil while non-sterile soil gave very low percentage of root colonization (Table 1).

Table 1: Effect of *G. Mosseae* Inoculation on the Biomass Yield of *A. Esculentus* (g plant⁻¹)

Treatment	%AMF	Root Dwt	Stem Dwt	Leaf Dwt	Fruit Dwt
STM ⁺	80.77	11.9 ± 0.3	23.0 ± 1.3	5.0 ± 0.2	17.2 ± 1.2
STM ⁻	-	10.5 ± 0.4	21.7 ± 1.3	3.0 ± 0.1	11.7 ± 1.6
NSTM ⁺	3.80	9.6 ± 0.4	13.5 ± 1.8	2.0 ± 0.5	5.6 ± 1.3
NSTM ⁻	2.59	8.0 ± 0.9	7.1 ± 0.8	2.0 ± 0.5	2.2 ± 0.7

*STM⁺: sterile soil inoculated; STM⁻: sterile soil uninoculated; NSTM⁺: non-sterile soil inoculated; NSTM⁻: non-sterile soil uninoculated. §Means of three replicates ± standard error of the mean.

Foliar Nutrient Yield

All inoculated plants irrespective of soil treatment consistently gave higher nitrogen and magnesium yield while phosphorus and potassium were lowest in inoculated plants growing in sterile soil (Table 2).

Table 2: Effect of *G. Mosseae* Inoculation on the Foliar Nutrient Yield of *A. Esculentus* (g kg⁻¹)

Treatment	N	P	K	Mg
*STM ⁺	§44.80	10.20	9.18	16.30
STM ⁻	42.00	11.90	10.80	1.90
NSTM ⁺	44.80	14.00	14.40	14.40
NSTM ⁻	29.40	12.10	10.40	1.90

*STM⁺: sterile soil inoculated; STM⁻: sterile soil uninoculated; NSTM⁺: non-sterile soil inoculated; NSTM⁻: non-sterile soil uninoculated. §Means of three replicates.

DISCUSSIONS

The higher biomass and fruit yield observed in inoculated plants is consistent with the findings of some earlier studies (El-Shaikh and Mohammed, 2009). This can be explained to be possibly due to the enhanced nutrient absorption

caused by the *Glomus mosseae* whose extra-radical hyphae must have increased root absorbing surface area leading to more nutrient absorption for enhanced growth. Also inoculation with arbuscular mycorrhizal fungus has been shown to increase chlorophyll content of leaves (Tanwar *et al.*, 2013). Thus increased chlorophyll would have resulted in increased photosynthesis and subsequently increased biomass production and accumulation. This suggestion is substantiated by the higher magnesium and nitrogen contents found in inoculated plants (Table 2).

The higher percentage AMF colonization of inoculated plants suggests a higher inoculum potential of the *G. mosseae*. However, the lower percentage of AMF colonization in inoculated non-sterile soil plants is in corroboration with some earlier studies (Okon *et al.*, 2007). This suppression of introduced AMF colonization in non-sterile soil could have been due to competition for space for colonization and infection site in the host by other soil borne pathogens (Cardoso and Kuyper, 2006); inhibition of the *G. mosseae* spores germination by the indigenous soil microorganisms (Linderman, 1992) or grazing by soil fauna on spores (Fitter and Sanders, 1992). Okon and Imuk (2011), has reported a high population of these nematodes in the soil of this area. The higher biomass and fruit yield in sterile soil plants than those in non-sterile soil can be attributed to the negative effect of soil borne pathogens in the non-sterile soil treatments which competed with the AMF thereby reducing their sprouting and colonization of the roots to enhance growth of the okra plants. Another possible reason for the reduced growth in non-sterile soil plants could have been due to root infection by the pathogens without much hindrance. AM fungi inoculation is known to protect plants against pathogens (Cardoso and Kuyper, 2006; Hemavani and Thippeswamy, 2014). Inoculation with AMF has been shown to improve plant growth even in the presence of parasitic nematodes (Okon and Imuk, 2011; Banuelos *et al.*, 2014). This they do by either competing with nematodes or other pathogens for infection sites or photosynthates thereby suppressing their growth (Yang *et al.*, 2014) or by improving the plant nutritional status as well as photosynthetic potential resulting in increasing resistance (Abdel-Fattah and Shabanam, 2002; Banuelos *et al.*, 2014).

Foliar nitrogen and magnesium yield were greatly increased in inoculated plants due to the ability of the *G. mosseae* to reach out beyond the nutrient depletion zone of the root to absorb these nutrients. Mycorrhizal colonization is known to increase root surface area for nutrient acquisition (Bhuvaneswari *et al.*, 2014). Phosphorus and potassium yield were however reduced in inoculated plants grown in sterile soil. This must have been due to dilution effect resulting from a higher biomass yield since the medium of growth was limiting being a pot experiment. Also unlike nitrogen and magnesium which are incorporated into chlorophyll molecules of the leaves, mobilization of more phosphorus and potassium to the fruits can also be a possible explanation for this disparity.

CONCLUSIONS

The results of this study has shown that inoculation with appropriate arbuscular mycorrhiza fungus can enhance the growth and yield of okra in a nematode infested soil such as found in the sandy loam soil of Cross River Basin in Nigeria.

ACKNOWLEDGEMENTS

The technical assistance of the staff of the department of Soil Science, University of Calabar is gratefully acknowledged.

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